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Effects of Low-Energy Laser Irradiation on Bone Remodeling During Experimental Tooth Movement in Rats

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Background and Objective: **Low-energy laser irradiation has many anabolic effects such as the acceleration of bone formation. However, its effects on tooth movement, performed by bone resorption and formation, have not been well characterized.**
Study Design/Materials and Methods: **A total of 10 g of orthodontic force was applied to rat molars to cause experimental tooth movement. A Ga-Al-As diode laser was used to irradiate the area around the moved tooth, and after 12 days, the amount of tooth movement was measured. Calcein was injected subcutaneously to label the newly formed alveolar bone for quantitative analysis. Immunohistochemical staining of proliferating cell nuclear antigen was performed to evaluate cellular proliferation. TRAPase staining was also performed to facilitate the identification of osteoclasts.**
Results: **In the laser irradiation group, the amount of tooth movement was significantly greater (1.3-fold) than that of the nonirradiation group in the end of the experiment. The amount of bone formation and rate of cellular proliferation in the tension side and the number of osteoclasts in the pressure side were all significantly increased in the irradiation group when compared with the nonirradiation group ($P < 0.01$).**
Conclusion: **These findings suggest that low-energy laser irradiation can accelerate tooth movement accompanied with alveolar bone remodeling. Lasers Surg. Med. 26:282–291, 2000** **© 2000 Wiley-Liss, Inc.**

Key words: Ga-Al-As diode laser; orthodontic tooth movement; alveolar bone remodeling; histomorphometry

INTRODUCTION

In orthodontic treatment, tooth movement is related to the response to applied orthodontic forces that cause remodeling of the periodontal tissues, especially alveolar bone. The typical 2- to 3-year treatment period is burdensome for patients, so it is very important to accelerate alveolar bone remodeling during treatment to abbreviate the time required. Recently, the acceleration of tooth movement caused by the injection of prostaglandin E_2 (PGE_2) [1], 1,25-$(OH)_2D_3$ [2], and PTH [3] in the area of the tooth where orthodontic force is applied has been investigated. However, many side effects have been reported. PGE_2 injection causes local pain and severe root resorption [4], and frequent injections of 1,25-$(OH)_2D_3$ or PTH may induce unfavorable general conditions. Recently, various biostimulatory effects of low-energy laser irradiation that involve wound healing [5–7], fibroblast [8–11] and chondral proliferation [12], collagen synthesis [13–16], and nerve regeneration [17] have been reported. In particular, the acceleration of bone regeneration by laser treatment has been a focus of contemporary research [18–22]. Therefore, if laser irradiation can cause the acceleration of bone remodeling, it may also have great potential benefit in abbreviating the orthodontic treatment period. We previously

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reported the stimulatory effects of low-energy laser irradiation on bone regeneration in a midpalatal suture during rapid maxillary expansion in rats [23] and also showed that low-energy laser irradiation significantly stimulated the number of bone nodules in a laser dose-dependent manner [24]. Furthermore, we have demonstrated that laser irradiation stimulates the cellular proliferation and differentiation of osteoblast lineage nodule-forming cells, especially in committed precursors, resulting in an increase in the number of differentiated osteoblastic cells as well as in bone formation [25]. On the other hand, tooth movement is caused by the reciprocal actions of bone formation in the tension side and bone resorption in the compression side, and the effect of laser irradiation on bone remodeling has yet to be well characterized.

In the present study, we investigated the effects of low-energy laser irradiation on tooth movement speed and bone remodeling during experimental tooth movement in rats. The effects of laser irradiation were evaluated quantitatively by measuring the amount of tooth movement, and bone formation, as well as the number of proliferating cell nuclear antigen (PCNA)-positive cells in the tension side and the number of osteoclasts in the compression side.

MATERIALS AND METHODS

Animals

A total of 48 male Wistar strain rats at 6 weeks old (180 ± 10 g) were used for the experiments, 24 rats for histomorphometric analysis and 24 for histologic examination. They were kept in the animal center of Nihon University School of Dentistry at Matsudo in separate cages, in a 12-hour light/dark environment at a constant temperature of 23°C and provided with food and water ad libitum. The health status of each rat was evaluated by daily body weight monitoring for 1 week before the start of the experiments.

Experimental Tooth Movement

For the histomorphometric analysis, 24 rats were divided into two groups of 12 rats each, to form the laser irradiation and nonirradiation groups.

All operations were carried out under general anesthesia by using an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). For mesial movement of the upper left 1st molar, the wire end of a 7.0-mm length of stainless steel, closed-coil spring (wire size: 0.005 inch, diameter: 1/12 inch, Accurate Sales Co. Chiba, Japan) was ligated with the maxillary 1st molar cleat by a 0.008-inch stainless steel ligature wire (Tomy International Inc. Tokyo, Japan). The other side of the coil spring was also ligated, with the holes in the maxillary incisors drilled laterally just above the gingival papilla with a #1/4 round bar, by using the same ligature wire (Fig. 1). The orthodontic force exerted by the appliance was 10 g in the beginning of the experiment. The force was determined based on a preliminary study that showed that the rat's upper 1st molar could be moved by orthodontic force without a decrease of body weight or hyalinized degenerative tissues [26]. Tooth movement was performed for 13 days (day 0 ~ 13).

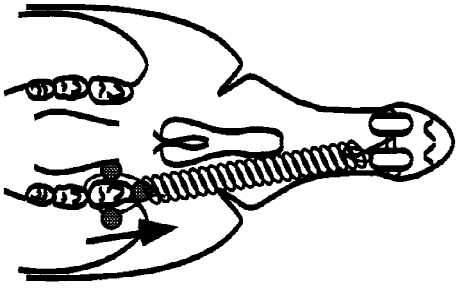

Fig. 1. Tooth movement and laser irradiation points. Upper left 1st molar was moved mesially by the closed coil spring (CCS) at 10 g of orthodontic force. Irradiation was performed by placing the optical fiber in direct contact with the tissue to prevent reflection of the laser beam onto the tissue surface. Shaded circles, laser irradiation points, arrow, tooth movement direction.

Measurement of Tooth Movement

To measure the amount of tooth movement, plaster models of the maxillae were made by using a silicone impression material (Dent Silicone-V, Shofu Inc. Kyoto, Japan) before (day 0) and after tooth movement (days 1, 2, 4, 12). The plaster model was placed under a substance microscope with the maxillary occlusal plane parallel to the object stage. The upper molar occlusal plane was determined by eliminating all of the cusps in the molars, except for the right third, left first, and left second molars. Occlusal standardized photographs were taken at ×16 magnification by using the fine measure. The amount of tooth movement was determined by the difference when measuring between the top of the mesiobuccal cusp of the first and second maxillary left molars, before (day 0) and after tooth movement (days 1, 2, 4, 12).

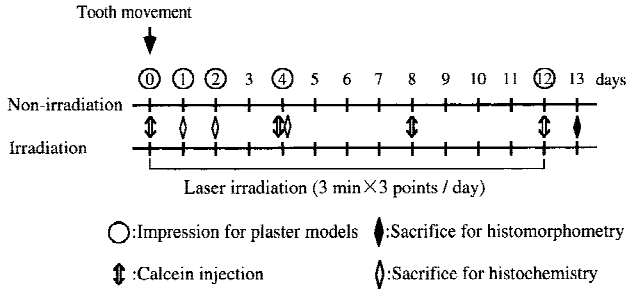

Fig. 2. Experiment time-schedule for each group.

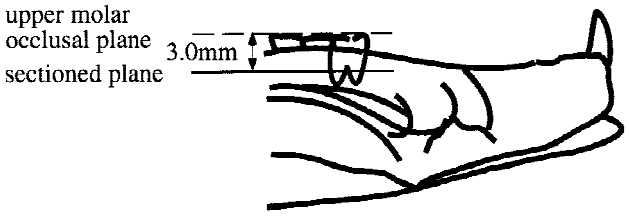

Fig. 3. Diagram of the datum plane of a maxillary horizontal section. The specimens were embedded in methyl methacrylate resin parallel to the upper molar occlusal plane. Each plastic embedded bone specimen was cut in a parallel plane 3.0 mm below the upper molar occlusal plane (dotted line).

Laser Irradiation

For the low-energy laser source, a gallium-aluminum-arsenide (Ga-Al-As) laser (Panalas 1000 prototype, Matsushita Industrial Equipment, Inc.) was used. The wave length was 830 nm and the output power was 100~700 mW, variable. Continuous waves at 100 mW output power conditions were used. These irradiation conditions were determined by previous experiments which showed that 5–10 minutes laser irradiation with a similar device (20–60J) stimulated bone formation in the tooth extraction socket or expanded midpalatal suture [23,27].

The laser beam was delivered by a 0.6-mm-diameter optical fiber, and irradiation was administered, under anesthesia, by placing the end of the optical fiber tip in contact with the mesial, buccal, palatal sides of the gingiva, located in the area of the moved upper left 1st molar. Irradiation was performed for 3 minutes at each point (total 9 minutes, 35.3W/cm^2) once a day (Fig. 1) on day 0 to day 12 (total 13 times). Total energy corresponding to a 9-minute exposure was 54.0 J, which is similar to the dose used in our previous study [23].

Bone Histomorphometry

The present experiment was performed following the time schedule shown in Figure 2. For alveolar bone labeling, all rats were injected subcutaneously with calcein (Dojin Co., Kumamoto, Japan) at a dose of 8 mg/kg on days 0, 4, 8, and 12. The rats were then killed on day 13 with an intraperitoneal injection of a lethal dose of sodium pentobarbital. The maxillae, including the irradiation sites were removed, fixed in ethyl alcohol overnight, and prepared for histomorphometric examination. The upper molar occlusal plane was determined as noted above. The bone specimens were immersed in Villanueva bone stain solution (5 mg/ml in 70% methanol; Maruto Instrument Co., Tokyo, Japan) for 72 hours and then embedded in methyl methacrylate resin, by using routine methods. Each plastic-embedded bone specimen was cut with a low-speed saw (Buehler Isomet, Buehler Ltd., Evanston, IL) in a line parallel to and 3.0 mm below the upper molar occlusal plane (Fig. 3). The specimens, taken from the 100-μm-thick section centered around the irradiation site, were prepared and then ground to a thickness of 30 μm.

The ground sections were photographed with a fluorescent microscope (Olympus BHS, Olympus, Tokyo, Japan) under ultraviolet light, and a division scale was also photographed at the same magnification for quantitative evaluation. An outline of the labeled bone was traced from the photographs. These traces were then entered into a personal computer (Macintosh G3, Apple Computer Inc.) by scanning with a flat-bed type scanner (EPSON GT-8000, SEIKO-EPSON, Tokyo, Japan). The newly formed mineralized bone areas were measured by using image analysis software (Ultimage Ver. 2.0, Graftek France, Voisins-Le-Bretonneux, France). Next, bone histomorphometry measurements were performed in the tension areas where one-quarter of the distal area was facing the mesial root (MR), as determined when linked with the center of the mesial root and the distobuccal root (DBR) of the left 1st molar (Fig. 4). The sectioned plane was determined 3.0 mm below the occlusal plane, with the result being 1.0 mm below the alveolar crest of the furcation. Because the tooth was tipping, bone formation would be greater in the area close to the top of the furcation. New bone formation areas were standardized by the following parameters: newly formed bone area (mm^2)/length of labeled alveolar bone margin (mm).

Histochemical Examination

For histologic examination, 24 rats were divided into six groups of four rats each as follows:

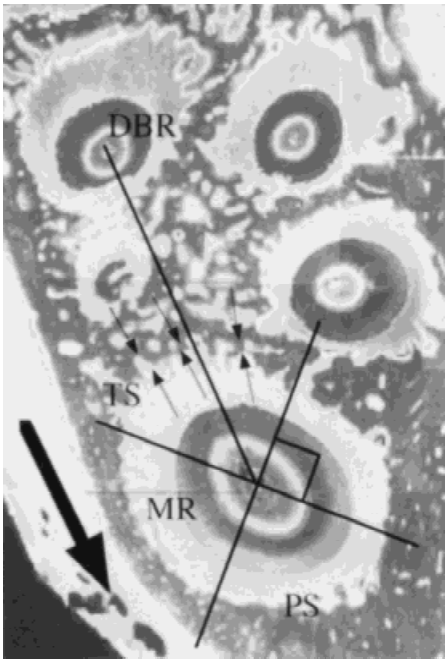

Fig. 4. Photograph of labeled alveolar bone taken under a fluorescent microscope. Bone formation was measured in the tension side (TS) quarter of the distal area facing MR. The number of the osteoclasts was measured in the pressure side (PS) quarter of the mesial area, opposite of the measuring TS. large arrow, tooth movement direction; MR, mesial root; DBR, distal buccal root; →←, new bone formation area in the tension side labeled by calcein.

the irradiation groups (day 0–1, day 0–2, day 0–4) and the nonirradiation groups (day 0–1, day 0–2, day 0–4). All rats were fixed by transcardial perfusion of 4% (w/v) paraformaldehyde in 0.1 mol/l phosphate buffered saline (PBS) solution. The maxillae, including the molars, were removed, immersed overnight in the same fixative at 4°C, and then rinsed with PBS solution. The tissue blocks were demineralized in 10% (w/v) ethylenediaminetetra-acetic acid for 14 days at 4°C and then embedded in paraffin. The embedded specimens were cut into 4.0-μm-thick sections, in a parallel plane 3.0 mm below the upper occlusal plane, the same area as for the ground sections.

To facilitate identification of osteoclasts, the tartrate-resistant acid phosphatase (TRAP) histochemical detection method elucidated by Cole et al. [28] was used with slight modifications. Briefly, 0.1 M acetate buffer solution (pH 5.0) was added to naphthol AS-BI phosphate (Sigma, St. Louis, MO). Fast red-violet LB diazonium salt, 10% $MnCl_2$, and 50 mM of L-(+)-tartaric acid were then added to the solution, which was then filtered and preheated to 37°C, before use. Deparaffinized slides were then incubated in the solution for 60 minutes in an incubator with the temperature adjusted to 37°C. The slides were then washed for 30 minutes in running water, counterstained with hematoxylin for 1 minute, air-dried at room temperature, and mounted with coverslips. Following the protocol of the previous study [29], eight sections at 32-μm intervals from 3.0 mm below the occlusal plane toward the apex were analyzed for TRAPase activity. The osteoclasts on the alveolar bone surface at the pressure side were counted in each section. Osteoclasts were defined as TRAPase-positive when there were more than three nuclear cells on the bone surface. The values from the eight sections were averaged for each rat. There were no significant differences found between the length of labeled alveolar bone edges in the sections of each rat ($P < 0.5$ Student's t-test).

To evaluate cell proliferation in the tension side, immunohistochemical staining by using the monoclonal antibody of PCNA (DAKO EPOS, PC-10) was performed. The sections for staining were the same parts used for measuring the area of bone formation. The deparaffinized slides were then incubated with 3% hydrogen peroxide in distilled water for 5 minutes in an incubator with the temperature adjusted to 37°C. The slides were washed with distilled water and placed in Tris-buffered saline (TBS) for 5 minutes, then covered with PCNA solution and incubated for 60 minutes. Immunoglobulin (DAKO EPOS Immunoglobulins) was used instead of PCNA solution for the negative control. Slides were washed in TBS for 5 minutes, incubated in chromogenic substrate solution (DAB: Dojin Chemicals, Kumamoto, Japan) for 10 minutes, and then counterstained with hematoxylin for 1 minute. The nature of the staining and distribution of PCNA immunoreactivity were evaluated by scoring the stained cells on the surface of the alveolar bone and in the periodontal ligament in the tension side on one-quarter of the distal area facing MR, in the same area as for bone histomorphometry. Results were standardized as follows: the number of PCNA-positive cells/the number of all stained cells (%).

Statistical Analysis

The values in each figure are represented as the mean ± SD for each group. Intergroup comparisons of the average values were conducted with Tukey's t-test for analysis of body weight change and Student's t-test for equal variance of the other comparisons. A value of $P < 0.05$ was considered to indicate a significant difference.

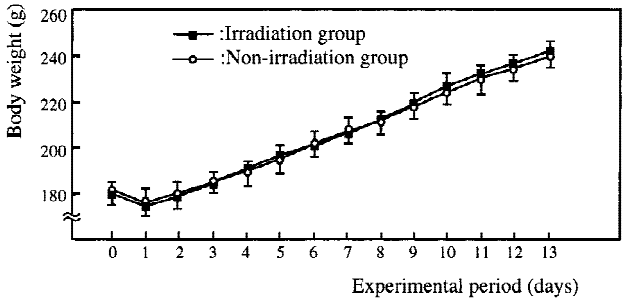

Fig. 5. Changes of body weight during the experimental period. There were no significant differences between the nonirradiation group and the irradiation group at each time point. Values are mean ± SD for ~16–24 rats.

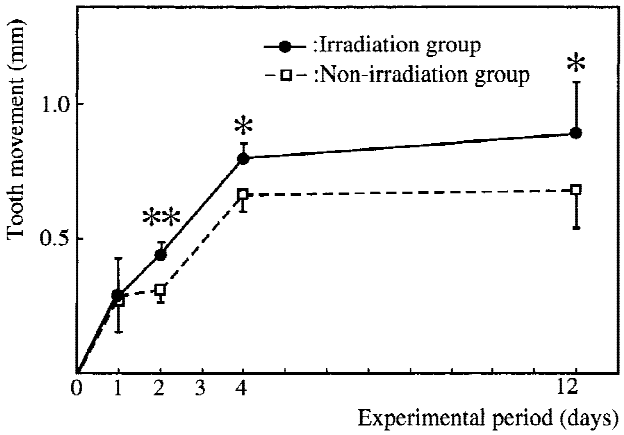

Fig. 6. Effect of laser irradiation on tooth movement. Significantly different from corresponding nonirradiation groups. Double asterisks indicate $P < 0.01$; single asterisk indicates $P < 0.05$. Values are mean ± SD for four rats.

RESULTS

Changes of Body Weight During the Experimental Period

The body weight of the rats in the nonirradiation and irradiation groups decreased transiently on day 1 and then recovered. No significant differences between the two groups were revealed by Tukey's t-test (Fig. 5).

Tooth Movement During the Experimental Period

The amount of tooth movement was significantly greater in the irradiation group on day 2 (1.4-fold), 4 (1.2-fold), and 12 (1.3-fold) compared with the nonirradiation group (Fig. 6).

Effects of Laser Irradiation on Bone Formation

The ground sections, stained by the Villanueva bone staining method, revealed clear bone labeling along the alveolar bone in the tension side area of the mesial root of the left 1st molar. The rate of new bone formation along the alveolar bone edges labeled by calcein was higher in the laser irradiation group than the nonirradiation group (Fig. 7). A quantitative evaluation by histomorphometric method showed that the development of newly formed mineralized bone in the tension side of the laser irradiation group was significantly accelerated (1.75-fold) compared with that of the nonirradiation group ($P < 0.01$) (Fig. 8).

Histochemical Examination for Osteoclasts

In the laser irradiation group, numerous osteoclasts were found on the alveolar bone surface at the pressure side on day 1 and day 2, whereas fewer osteoclasts were observed in the pressure side in the nonirradiation group. Figure 9 shows TRAP histochemical staining sections in the pressure side on day 2.

In the quantitative evaluation, the increase in the number of osteoclasts in the irradiation group on day 2 was significant in comparison with each corresponding nonirradiation group. The number of osteoclasts significantly increased, time-dependently, from day 0 to day 2 in both groups (one-way analysis of variance, $P < 0.01$). However, a decrease on day 4 was seen in both groups (Fig. 10).

Immunohistochemical Examination for PCNA

PCNA-positive cells were found in the periodontal ligament on the tension side in both the irradiation and nonirradiation groups. However, the number of PCNA-positive cells seemed to be greater in the irradiation group than in the nonirradiation group (Fig. 11). In the quantitative evaluation, the rate of PCNA-positive cells was found to be significantly increased (1.3-fold) in the irradiation group on both day 1 and day 2 compared with the nonirradiation group ($P < 0.01$) (Fig. 12).

DISCUSSION

In the present study, we clearly demonstrated the stimulatory effects of low-energy Ga-Al-As diode laser irradiation on experimental tooth movement. The effect was 1.3-fold greater than in the nonirradiation group and stimulation

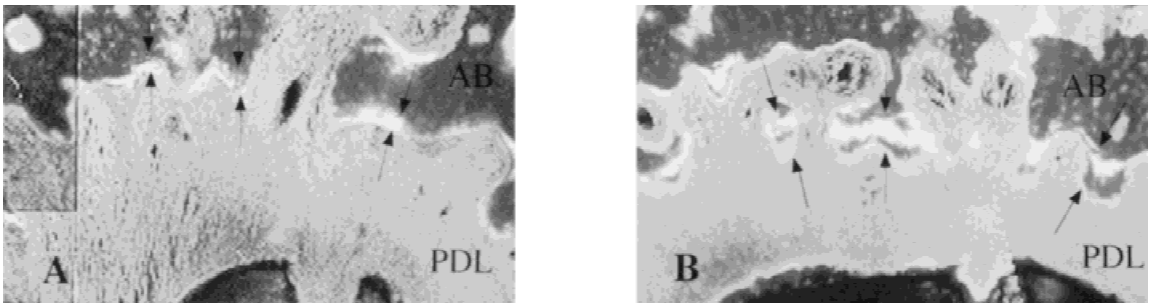

Fig. 7. **A,B:** Comparison of new bone formation area with or without application of laser irradiation. Bone formation (day 0–12) rate (→←) was higher in the laser irradiation group (B) than in the nonirradiation group (A). PDL, periodontal ligament; AB, alveolar bone.

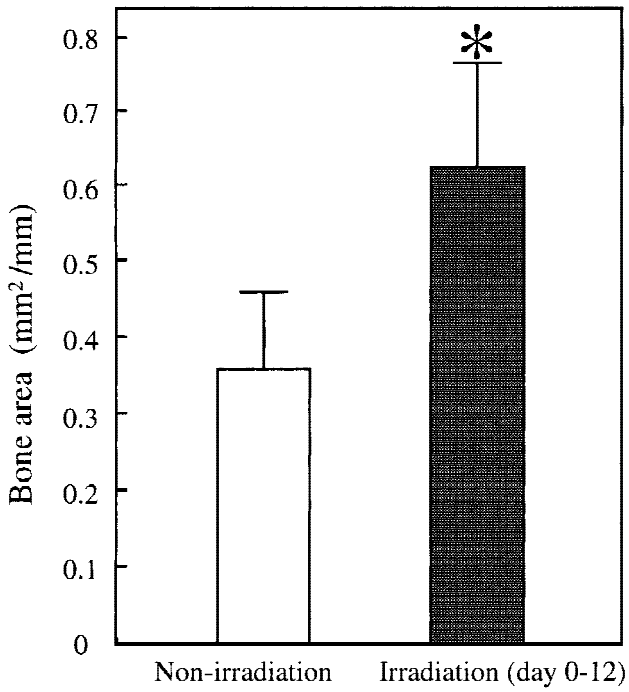

Fig. 8. Effects of laser irradiation on newly formed mineralized bone area in the tension side during mesial movement of the upper left 1st molar. The asterisk indicates a significant difference from nonirradiation group ($P < 0.01$). Values are mean ± SD for 12 rats.

of tooth movement was accompanied with enhanced alveolar bone remodeling.

The speed of tooth movement is greatly dependent on the speed of bone remodeling. The amount of tooth movement on day 1 was caused by distortion of the periodontal ligament, before bone remodeling began (Fig. 6), thus, the amount of tooth movement in both groups was the same. In the nonirradiation group, there was almost no additional tooth movement from day 1 to day 2, because bone remodeling was not active enough to move the tooth, even though the number of osteoclasts tended to increase on day 1 (Fig. 10). Thereafter, the tooth started to move accompanied by bone remodeling. In contrast, tooth movement occurred in the irradiation group from day 1 to day 2, but the difference in amount of tooth movement in both groups on day 2 did not change during the experimental period. Therefore, it is quite likely that laser irradiation increased tooth movement at an early stage (day 1–2). This conclusion may be supported by the greater increase in the number of osteoclasts in the compression side in the irradiation group compared with the nonirradiation group on day 2. It is reported that the first step in bone remodeling is resorption by osteoclasts [30], and that osteoclast functions are estimated by stem cell division [31], chemotaxis of preosteoclasts [32], recognition [33], attachment [34], or fusion [35]. In the present study, the number of multinuclear osteoclasts increased 1.6-fold in the irradiation group as compared with that in the nonirradiation group, suggesting that laser irradiation may stimulate the fusion of mononuclear macrophages to mature osteoclasts. It has been reported that local administration of 1.25-$(OH)_2D_3$ accelerates experimental tooth movement [36] and also induces an increase in the number of osteoclasts on the bone surface [37,38]. It was also reported in an in vitro study that 1.25-$(OH)_2D_3$ treatment to mononuclear macrophages stimulates the fusion of these cells, resulting in an increase in multinuclear osteoclasts [39]. Laser irradiation, therefore, may have effects similar to 1.25-$(OH)_2D_3$ administration on mononuclear macrophages and may accelerate their fusion into fully functional multinuclear osteoclasts. It may be interesting to elucidate the effect of laser irradiation on osteoclast formation in

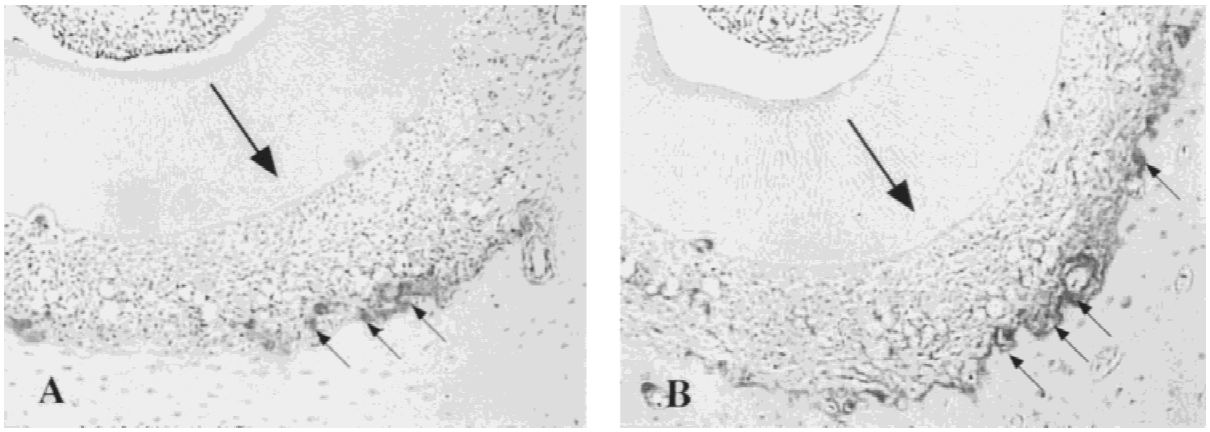

Fig. 9. **A,B:** Comparison of osteoclast distribution on day 2 with or without application of laser irradiation. Many TRAPase-positive multinuclear cells were found on the alveolar bone surfaces in the laser irradiation group (B) compared with the nonirradiation group (A). Large arrows, tooth movement direction; small arrows, TRAPase-positive multinuclear osteoclasts.

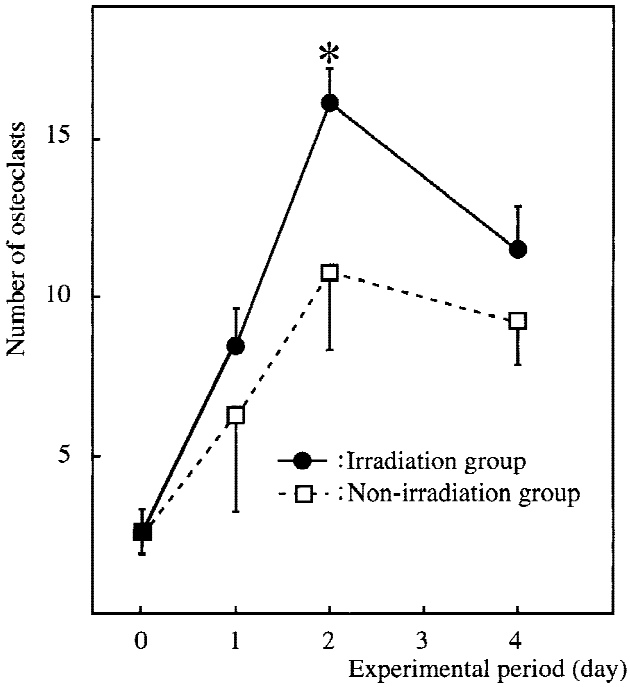

Fig. 10. The number of osteoclasts in the pressure side of the irradiation and nonirradiation groups. The asterisk indicates a significant difference from corresponding nonirradiation group ($P < 0.01$). Values are mean ± SD for four rats.

vitro. Moreover, the effects of laser irradiation on other osteoclast functions such as stem cell division, chemotaxis, recognition, or attachment should be also studied.

In an investigation of bone formation in the tension side, newly formed mineralized bone area was found to be accelerated 1.7-fold in the irradiation group compared with that of the nonirradiation group. Because each calcein labeling line could not be distinguished clearly in the present experimental conditions, we could not estimate which part of the bone area was accelerated by laser irradiation during the period from day 0 to day 12. However, when considering the acceleration of tooth movement by laser irradiation from day 1 to day 2, it is possible to conclude that laser irradiation may stimulate bone formation in this early stage of tooth movement. We have previously reported that low-energy laser irradiation applied to a midpalatal suture during rapid maxillary expansion in rats accelerated bone regeneration 1.4-fold in the bone edges of the suture and that the early period after expansion (day 0–3) was the most effective for acceleration [23]. This previous study may support the results of the present study. We have also reported, by using an in vitro experimental model, that laser irradiation caused an increase in the number of more differentiated osteoblastic cells and bone nodule formation, by stimulating the cellular proliferation of osteoblast lineage nodule-forming cells and cellular differentiation at an early stage of cell culture [25]. Because the stimulatory effect of bone formation by laser irradiation in the tension side in the present experiment may have been influenced by cellular proliferation, differentiation, matrix formation and/or mineralization, immunohistochemical staining by using the monoclonal antibody of PCNA was performed to elucidate the effect of laser irradiation on osteoblasts and fibroblasts. Immunohistochemical staining for PCNA in paraffin-embedded sections has been reported to be a useful method for the

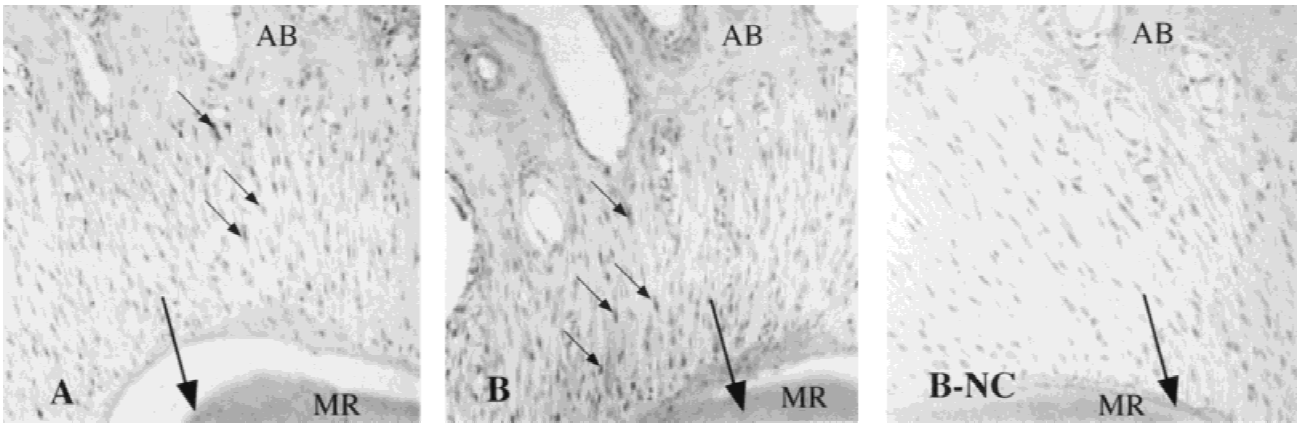

Fig. 11. Comparison of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactivity in the tension side with or without application of laser irradiation on day 2. More PCNA staining cells were found in the laser irradiation group (**B**) compared with the nonirradiation group (**A**). No PCNA staining cells were found in the negative control irradiation group (B-NC). AB, alveolar bone; MR, mesial root; large arrows, tooth movement direction; small arrows, PCNA(+) cells.

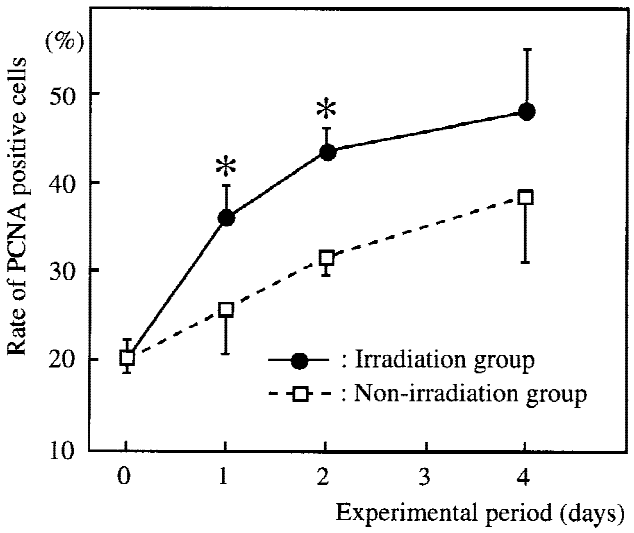

Fig. 12. The rate of PCNA-positive cells (the number of PCNA-positive cells/ the number of all the cells) in the tension side of the irradiation and nonirradiation groups. The asterisks indicate a significant difference from corresponding nonirradiation group($P < 0.01$). Values are mean ± SD for four rats.

evaluation of cell-proliferative activity [40]. PCNA protein is a cell cycle-related nuclear protein that is maximally elevated in the late G1 and S phases of proliferating cells. Our data indicated that the rate of PCNA-positive cells in the periodontal ligament was significantly higher in the irradiation group than the nonirradiation group from day 1 to day 2. These results are in general agreement with the results of our previous study [25] that demonstrated cellular proliferation stimulation in the early stage of cell culture by laser irradiation.

We found no significant differences in the body weight of the rats during the experimental period between the laser irradiation and nonirradiation groups. Furthermore, inflammation or burning was also not found around the laser irradiation points. Saito and Shimizu [23] also reported that Ga-Al-As diode laser irradiation had no influence on the bodies of tested rats. The Ga-Al-As diode laser is known to be a high tissue-penetration laser, as hemoglobin and water have a low coefficient of absorption [41]. A previous study revealed that approximately 50% of the diode laser beam (60 mW) penetrates to a depth of 1.0 mm in human and bovine mandibular cortical bone [42]. Because we used a power of 100 mW and the optical fiber tip was in contact with the gingiva so as not to reflect the laser light, it is most likely that an adequate dose of laser light reached the periodontal ligament and alveolar bone to accelerate bone formation.

In conclusion, low-energy Ga-Al-As diode laser irradiation-stimulated tooth movement accompanied with an acceleration of alveolar bone remodeling, as indicated by increases in the number of osteoclasts, cellular proliferation of periodontal ligament cells, and mineralized bone formation. Although further studies such as the effects of different irradiation dosages, the prolonged use of laser irradiation, or both, on tooth movement and bone remodeling are still required for clinical use consideration, the introduction of laser therapy at an early stage of tooth movement in orthodontic treatment seems feasible, and may be of great therapeutic benefit to abbreviate the treatment period.

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